

Speedy™ Human/Cynomolgus Monkey IL-17A One-Step ELISA Kit

Datasheet

Please read it entirely before use

Catalogue Number: SE50331

Size: 96T

Sensitivity: 0.3 pg/mL

Range: 62.5-4000 pg/mL

Usage: For the quantitative detection of human/cynomolgus monkey IL-17A concentrations in serum, plasma and cell culture supernatant.

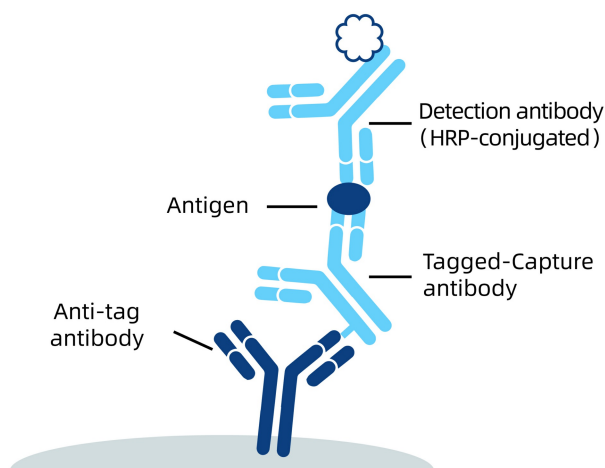
This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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1. Background

IL17A, also named as IL-17, is a proinflammatory cytokine. IL-17, synthesized only by memory T cells and natural killer cells, has pleiotropic effects, mainly in the recruitment and activation of neutrophils. This cytokine regulates the activities of NF-kappaB and mitogen-activated protein kinases. This cytokine can stimulate the expression of IL6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide (NO). High levels of this cytokine are associated with several chronic inflammatory diseases including rheumatoid arthritis, psoriasis and multiple sclerosis. The IL-17 receptor is a type I transmembrane protein, that is widely expressed on epithelial cells, fibroblasts, B and T cells, and monocytic cells. In psoriatic skin lesions, both Th17 cells and their downstream effector molecules, e.g. IL-17 and IL-22, are highly increased.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, <https://www.ptgcn.com/products/elisa-kits/>.
- 3.8 Microplate thermostatic shaker.

4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 8000 pg/bottle; lyophilized	2 bottles	
Capture Antibody (100×) - 60 uL/vial*	1 vial	
Detection antibody, HRP-Conjugated (100×) - 60 uL/vial*	1 vial	
Additional Diluent AT-50331 - 6 mL/bottle; Only for human serum and plasma samples.	1 bottle	
Sample Diluent PT 1 - 30 mL/bottle; For human serum and plasma samples	1 bottle	
Sample Diluent PT 4B1 - 30 mL/bottle; For cell culture supernatant samples.	1 bottle	
Detection Diluent PT PB1 - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

- 6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 uL 100X capture antibody + 50 uL 100X Detection Antibody, HRP-conjugated + 4,900 uL Detection Diluent. Mix gently but thoroughly.

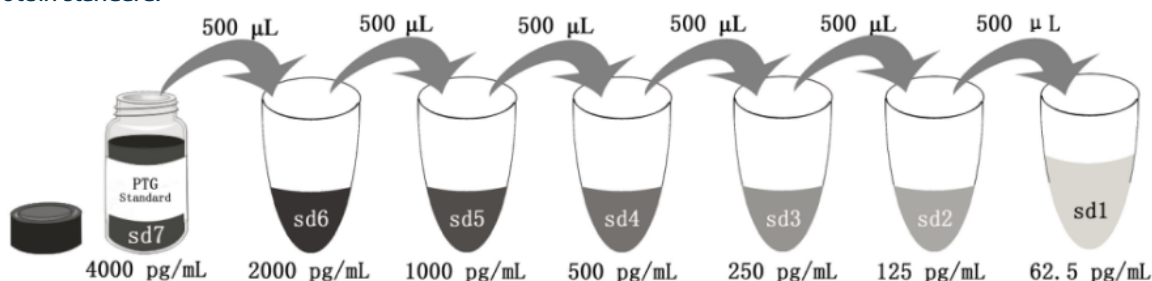
7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 uL of undiluted sample was added per well.

Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 is recommended for human serum and plasma; 1:2 or 1:4 is recommended for cell culture supernatant.

7.4 Standard Serial Dilution:

For human serum and plasma, add 2 mL Sample Diluent PT 1 in protein standard. For cell culture supernatant, add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1 or PT 4B1	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group:

For human serum or plasma, add 25 µL of Additional Diluent to the appropriate wells (No need incubation and wash);

For cell culture supernatant, no need to add Additional Diluent, directly follow the next step.

8.3 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.4 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.5 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

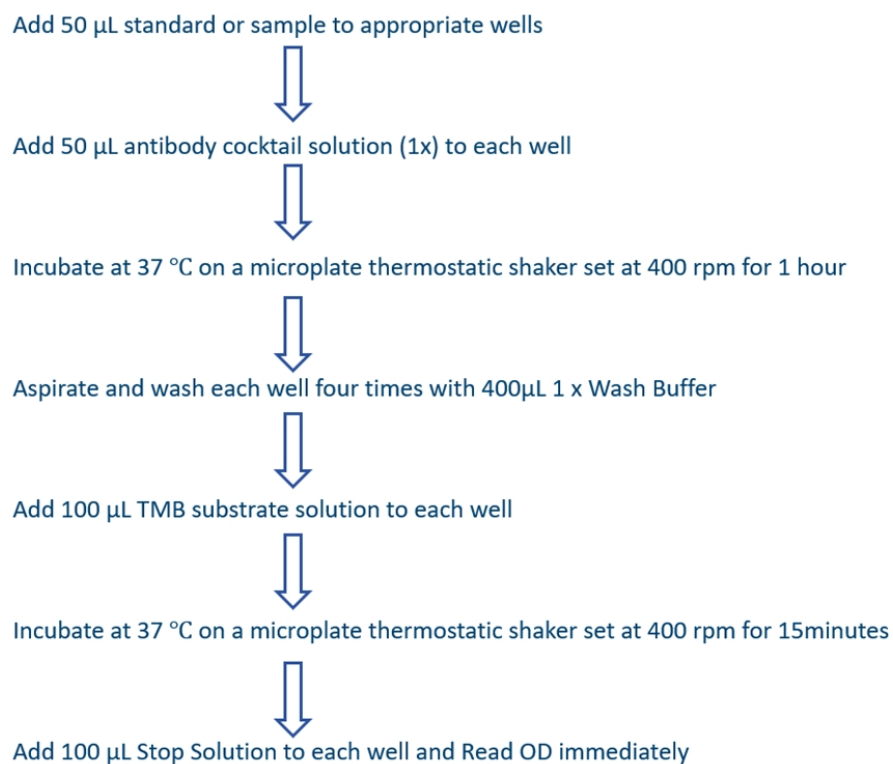
8.6 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.7 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.8 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.9 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve-fit (4-PL) analysis is recommended. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

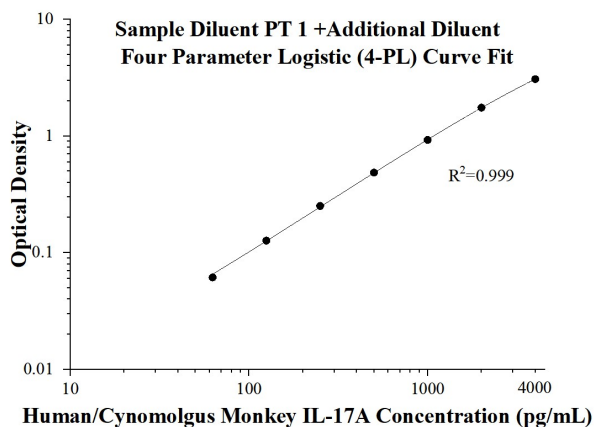
Procedure summary



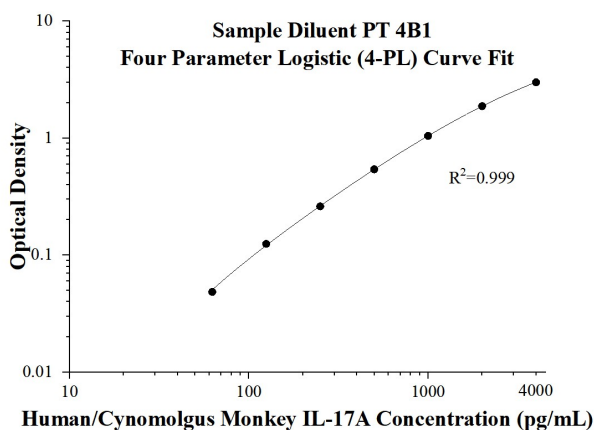
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.015 0.0148	0.0149	-
62.5	0.0762 0.0762	0.0762	0.0613
125	0.1452 0.1383	0.1418	0.1269
250	0.2677 0.2658	0.2668	0.2519
500	0.5159 0.4872	0.5016	0.4867
1000	0.9506 0.9306	0.9406	0.9257
2000	1.7946 1.7387	1.7667	1.7518
4000	3.1418 3.0496	3.0957	3.0808



(pg/mL)	O.D	Average	Corrected
0	0.0702 0.0647	0.0675	-
62.5	0.116 0.1157	0.1159	0.0484
125	0.1915 0.1928	0.1922	0.1247
250	0.323 0.3341	0.3286	0.2611
500	0.6057 0.6124	0.6091	0.5416
1000	1.109 1.1153	1.1122	1.0447
2000	1.9397 1.9439	1.9418	1.8744
4000	3.0628 3.0783	3.0706	3.0031

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	8	1,778.0	43.8	2.5	1	16	1,788.7	62.0	3.5
2	8	464.3	21.7	4.7	2	16	461.5	16.9	3.7
3	8	232.6	11.6	5.0	3	16	232.1	10.5	4.5

9.3 Recovery

The recovery of human/cynomolgus monkey IL-17A spiked to three different levels throughout the range of the assay was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	74	71-78
Cell culture supernatant	1:2	115	111-117
	1:4	113	111-116

9.4 Sample values

Human plasma - Fifteen plasma samples from volunteers were evaluated for IL-17A in this assay. All samples measured less than the lowest standard, 62.5 pg/mL. No medical histories were available for the donors used in this study.

Cell culture supernatant:

Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μ g/mL streptomycin sulfate. Cells were stimulated with 10 μ g/mL PHA for 3 days. Aliquots were removed on days 3 and assayed for levels of IL-17A.

Condition	Day 3 (pg/mL)
Unstimulated	ND
Stimulated	544.8

ND*=Non-detectable

Cynomolgus monkey peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μ g/mL streptomycin sulfate. Cells were stimulated with 10 μ g/mL PHA for 5 days. Aliquots were removed on days 5 and assayed for levels of IL-17A.

Condition	Day 5 (pg/mL)
Unstimulated	ND
Stimulated	2,559.2

ND*=Non-detectable

9.5 Sensitivity

The minimum detectable dose of human IL-17A is 0.3 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, human plasma samples were spiked with high concentrations of IL-17A and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatant samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatant
1:2	Average% of Expected	88	100
	Range (%)	86-91	-
1:4	Average% of Expected	95	91
	Range (%)	93-96	86-96
1:8	Average% of Expected	102	100
	Range (%)	100-103	89-110
1:16	Average% of Expected	102	93
	Range (%)	100-103	90-96

9.7 Specificity

This kit specifically recognizes native and recombinant human/cynomolgus monkey IL-17A.

10. References

1. T. Mabuchi. et al. (2012). J Dermatol Sci. 65: 4-11.
2. X. Song. et al. (2013). Cytokine. 62: 175-182.
3. E. Fitch. et al. (2007). Curr Rheumatol Rep. 9: 461-467.
4. provided by RefSeq, Jul 2008.